Asian Journal of Pharmaceutical Research and Health Care, *Vol 10(1), 1-9, 2018*

ISSN (Online) : 2250-1460
DOI: 10.18311/ajprhc/2018/19587

Comparative Experimental Studies of Few L-Type and T-Type Ca^{2+} Channel Blockers Against *In-Ovo* and *In-Vitro* Models of Angiogenesis

Vijay R. Chidrawar[1*] and Mohd. Imran[2]

[1]Department of Pharmacology and Toxicology, Northern Border University, Rafha, Kingdom of Saudi Arabia; vijay_pharmacology@yahoo.com, vijay_pharmacology@nbu.edu.sa
[2]Department of Pharmaceutical Chemistry, Northern Border University, Rafha, Kingdom of Saudi Arabia; imran.pchem@gmail.com

Abstract

Angiogenesis is the development of new blood vessels from pre-existing one. The ion-channels on endothelium plays vital role in cell proliferation and related angiogenesis. We aimed to investigate the effects of L-type (Verapamil and Diltiazem) and T-type (Ethosuximide) Calcium Channel Blockers (CCBs) on neovascularization. The effects on neovascularization were investigated by *in-ovo* (CAM, Chick Chorioallantoic membrane) and *in-vitro* (aortic ring assay) methods. Each test drug was tested for at least 3 doses and the anti-angiogenic effect was compared with Suramin as standard and normal control groups. Various vital parameters were recorded during the experiment like the number of blood capillaries, sprouts formation, angiogenic score etc. The L-type Ca^{2+} channel blockers Verapamil at the dose of 50µM, 110 and 220 µM/disk has shown significant ($p < 0.001$) reduction in the number of branching points in CAM assay. For the further confirmation, angiogenic activity was evaluated *in vitro* by rat aortic ring assay method; the area of sprouts was reduced by the medium and high dose of verapamil. Diltiazem has demonstrated modest anti-angiogenic activity by both the models, whereas T-type calcium channel blocker ethosuximide has not shown any effect on the neovascularization. Among all the tested drugs verapamil has shown the promising anti-angiogenic property. Thus verapamil **and** diltiazem may have anti-angiogenic activity defines novel beneficial effects in angiogenic mediated pathological conditions in addition to their main indications in cardiovascular complications.

Keywords: Angiogenesis, CAM Assay, L-Type Ca^{2+} Channel Blockers, Rat Aortic Ring Assay, T-type Calcium Channel Blocker

1. Introduction

Blood capillaries play a vital role in governing normal physiological process such as wound healing, during embryonic development and corpus luteum formation endometrium formation etc[1]. In addition, it involves in many pathological processes like diabetic retinopathy, arthritis, inflammation, and many other conditions[2,3]. Angiogenesis is responsible for the pathological progress and metamorphosis of tumor[4]. The newly developed blood capillaries supplement cancer growth by supplying nutrients, oxygen and by taking away waste products. Angiogenesis also supports Metastasis[5].

Angiogenesis is sequential and highly co-ordinated system involves several steps including basement membrane proteolysis, migration of individual endothelial cells, and endothelial cell proliferation[6].

Inhibition of angiogenesis and arresting development of blood capillaries has emerged as a promising strategy for the treatment of cancer[2]. Many researchers are

*Author for correspondence

attracted to develop anti-angiogenic drug and identified few lead molecules which are in phase II and phase III clinical trials[7].

The angiogenesis involves closely related sequential interdependent process including basement membrane proteolysis, migration of individual endothelial cells, and endothelial cell proliferation[6].

Cell proliferation and angiogenesis are complex mechanisms coordinated by several proteins related to Ca^{2+} signaling in various parts of the cell. In the 1970's, primary experimental evidence suggested that cell proliferation and angiogenesis might be regulated by Ca^{2+} ions[8,9]. This thought was later clarified when the involvement of Ca^{2+} channels was observed in normal and pathological cell proliferation[10]. Precisely, it was observed that normal cells require high external Ca^{2+} concentrations[11–14] to trigger cell proliferation while tumor cells demand much less Ca^{2+} influx[9–12]. In light of these evidence, various types of T-type (Verapamil, Diltiazem) and L-type (Ethosuximide) calcium channel blockers were screened and compared their effects by using Chick Chorioallantoic Membrane (CAM) assay and (*in ovo*) Rat aortic ring assay (*in vitro*) models of angiogenesis.

2. Material and Methods

2.1 Materials

Chemical and lab wares: Diltiazem, Verapamil, Nifedipine, Flunarizine dihydrochloride and Ethosuximide was purchased from Santa Cruz, USA. Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix was purchased from Al Genome International scientific and laboratory products Dubai, UAE. All the chemicals used in the research are of AR grade.

Equipment: Incubator, high resolution digital camera (Cyber-Shot 6.0, Sony, Tokyo, Japan), micropipettes, oven etc.

Experimental animal and maintenance: A total of 03 Wistar albino rats weighing in between 150-200 g were purchased from central animal house of Northern Border University, Saudi Arabia. The animals were maintained at a controlled temperature (22–25° C, 45% humidity) on a 12:12-h dark–light cycle. The animals were provided with adequate standard diet and ecofriendly conditions throughout the experiment. All the experiments were carried out between 9:00-16:00 hours. National Committees of BioEthics (NCEB) guidelines projected by Saudi Arabia were strictly followed and all the studies were approved by the Local Committee of BioEthics (LCEB), (Ref: 8/38/A).

2.2 Chick Chorioallantoic Membrane Assay

Fertilized chicken eggs were collected at day '0'. They were grouped randomly into various groups as per the treatment schedule. The eggs were incubated at 37^{0}C. On the 3rd day, 2-3 ml of albumin was withdrawn from the narrow end of the egg by creating a small hole. Then after window was sealed with cellophane tape and again incubated. On the 7th day of incubation a square window was created in the shell and filter paper disk (3mm×3mm×1mm) was implanted on top of the CAM tissue (yellowish forge wheel like structure) membrane. The test drug was initially applied on the filter paper disk and dried in the oven after drying these disks were placed on the CAM tissue. The group I (normal control) was impregnated with distilled water; the tests drugs were loaded on the filter paper at three doses as shown in the Table 1 and suramin was used as a standard. The eggs were property sealed again by using cellophane tape and kept it back to the incubator and they were incubated undisturbed till day 14. On the 14th day of incubation, the eggs were taken out from the incubator and the CAM tissues directly beneath each filter paper was resected with the help of forceps[15].

The anti-angiogenic effect of the test drugs was quantified by comparing the quantity of blood capillaries mainly under the area of the disk (where the drug was loaded) by capturing the images before and after treatment. The scale of 0-2 was used for the rating[16,17].

In this assay the mortality rate is around 30-40 % so to get at least 8-10 viable eggs in each group, 18 eggs kept for each sample.

The average score for each test dose was calculated and the interpretation of anti-angiogenic effect was done as follows:

Average score < 0.5 = no anti-angiogenic effect (inactive).

0.5 ≤ average score ≤ 1 = weak anti-angiogenic effect.

1 < average score < 1.5 = good anti-angiogenic effect.

Average score ≥1.5 = strong anti-angiogenic effect.

For every test drug, 10-15 eggs were used in parallel. For the evaluation of the effects a scoring system was used[16].

Briefly, the score obtained from above equation was assigned as follows:

$$Average\ score = \frac{\text{Number of (score 2)} \times 2}{Total\ number\ of\ eggs\ (\text{score } 0, 1, 2)} + \frac{\text{Number of (score 1)} \times 1}{Total\ number\ of\ eggs\ (\text{score } 0, 1, 2)}$$

Score system of anti angiogenic activity:

Score < 0.5 % = no anti-angiogenic effect

Score 0.5 to 0.75 % weak anti-angiogenic effect

Score > 0.75 to 1 = good anti-angiogenic effect

Score > 1 % very good anti-angiogenic effect.

2.3 Rat Aortic Ring Assay

Aortic Ring Assay (in vitro) method is one of the popular methods used for the screening of pro-angiogenic and anti-angiogenic activity. A healthy male Wistar rat weighing in between 200-210 gm sacrificed by CO_2 over dose thoracic cage was opened and the surrounding organs were detached. Thoracic aorta was isolated and immediately it was transferred to cold PBS supplied with aeration. While isolating much traction was avoided to maintain the integrity of the aorta. Aorta was cut into 1mm ring size and washed with DMEM (Dulbecco's modified Eagle's medium)[18].

These rings were placed in the 24 well plates with 150µl of corning Matrigel without growth factor. Initially, matrigel was stored at -20°C to liquefy and the micropipettes tips also were kept at -20°C to avoid any gelling effect of tips on the matrigel. Rings were completely immersed in matrigel and were allowed to polymerize for 1-2 hours at 37°C and then challenged to hypoxic conditions for 2 hours. This hypoxic condition stimulates the formation of sprouts from the rings. This was reoxygenated for 7 days and the abundance of blood vessels was quantified. Doses were selected based on the IC_{50} values and from earlier literature. Three doses were selected for each test drug; Verapamil (55, 110, 220 µM)[19], Diltiazem 1, 10 100 µM[20], ethosuximide (0.3, 0.6 and 1.2 mM)[21]. Dosing schedule is mentioned Table 1. Development of microcapillary and length of the capillaries formed was measured under the microscope at 400 X magnification using stage micrometer.

2.4 Statistical Analysis

Data were expressed as means ± standard deviation. Statistical analysis was performed using one-way ANOVA followed by Dunnett test. p = 0.05 level of significance. All the statistical analysis was done by using graph-pad Prism 7.

Table 1. Treatment schedule L-type and T-type calcium channel blockers for anti-angiogenic activity

Group No	Treatment	Dose per Ring
1.	Control	Distilled water
2.	Standard Drug (Suramin)	38.54 µM
3.	Verapamil (Low dose)	55 µM
4.	Verapamil (Medium dose)	110 µM
5.	Verapamil (High dose)	220 µM
6.	Diltiazem (Low dose)	1 µM
7.	Diltiazem (Medium dose)	10 µM
8.	Diltiazem (High dose)	100 µM
9.	Ethosuximide (Low dose)	0.3 mM
10.	Ethosuximide (Medium dose)	0.6 mM
11.	Ethosuximide (High dose)	1.2 mM

3. Results

3.1 Chick Chorioallantoic Membrane (CAM) Assay

Not all the test drugs caused the inhibition of vessel development in the CAM Figure 1. depicts maximum inhibitory effects of verapamil and standard drug Suramin on the number of branching points. No changes in the growth patterns of the micro capillaries were observed by the ethosuximide treated groups at all the selected doses. Verapamil at medium and high dose, significantly ($p<0.001$) inhibited number of branching points by 17.3 and 7 at 110µM and 220µM concentration compared to control group. Diltiazem treated groups have shown slight but significant ($p<0.001$) reduction in the number of branching points by 30.66 and 24.16 compared to normal control group. (Figure 1)

Test drug Verapamil also have shown very good angiogenic score in the range of 1 to 1.5. In fact, Verapamil has shown significant ($p<0.001$) dose-dependent increase in the angiogenic score at all the three doses i.e., 0.5, 0.75 and 1.3 at the concentration of 55, 110 and 220 µM/disk respectively. (Figure 2)

Ethosuximide, a T-type calcium channel blocker has not demonstrated any inhibitory effects on vessel growth in the CAM.

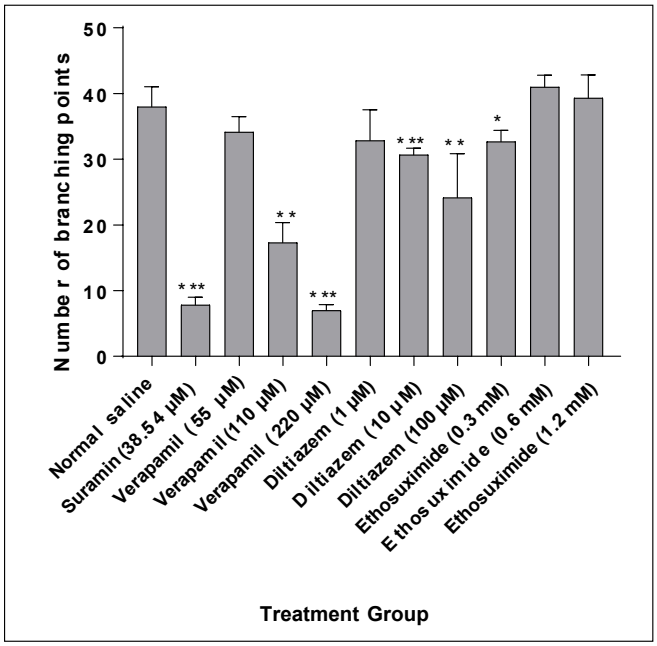


Figure 1. Comparative effect of L-type and T-type calcium channel blockers on the number of branching points of the blood vessels by CAM assay of angiogenesis.

It's essential to take note of that the evaluation strategy utilized does not recognize recently developed micro vessels (after utilization of the medication containing plates on the CAM) and those already present on the seventh day. Hence, 100% diminution of vascular thickness isn't conceivable by this assay and the number of branching points 17.3 and 7 by the medium and high of verapamil can be considered as the maximum achievable limit.

Interestingly, no micro vessels were detected on the CAM under the disc containing 220 µM of verapamil, suggesting that this compound not only hinder the formation of new micro-vessels but also destroy or regression of existing capillaries.

3.2 Aortic Ring Assay

The CAM experiment demonstrated that verapamil could inhibit angiogenesis. To further determine whether verapamil inhibited angiogenesis *ex vivo*, the effect of verapamil and other test drugs on the sprouting of microvessels from the aortic rings was carried out. As shown in Figure 3, compared to the control, sprouts around the ring

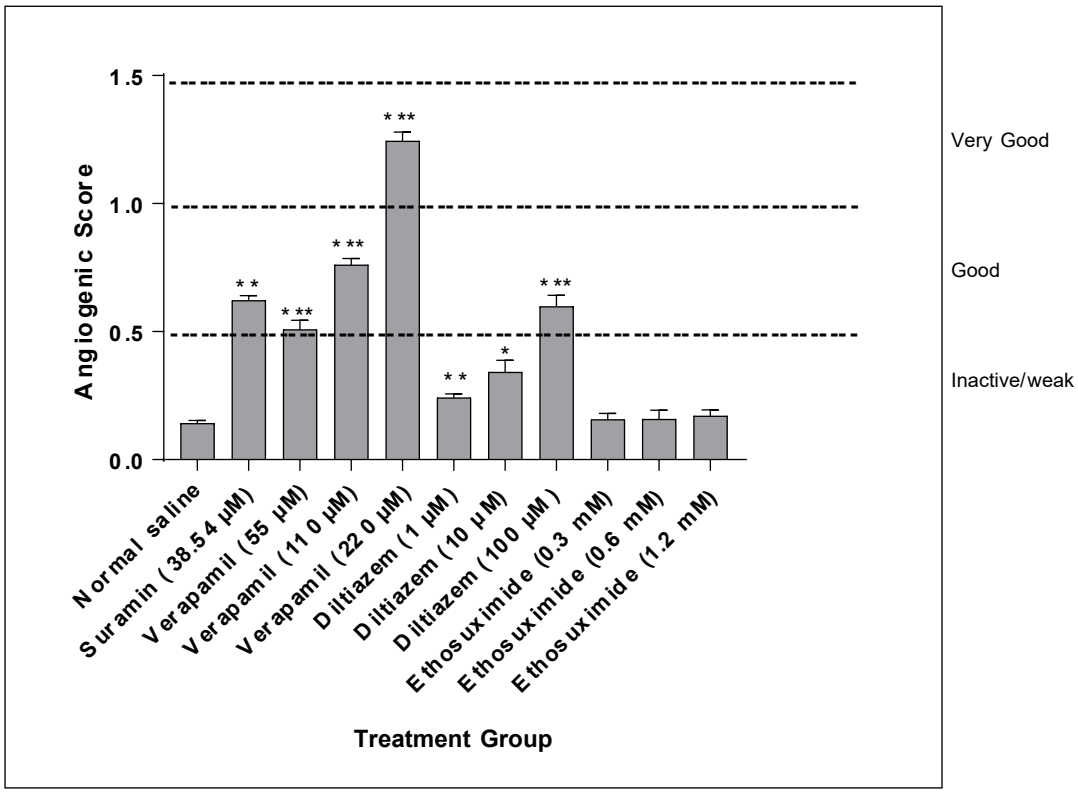


Figure 2. Comparative Anti-angiogenic effect (CAM-assay) of L-type, T-type Calcium Channel blockers and positive control.

treated with verapamil (55, 110 and 220 μM) and standard Suramin (38.54 μM) the sprouts around the rings were shorter. These data suggested that verapamil could inhibit the sprout length and density in the dose-dependent manner. Diltiazem also showed significant ($p<0.01$) dose-dependent decrease in the number and the length of microvessel sprouting compared to the normal group but the potency is less as compared to verapamil-treated groups.

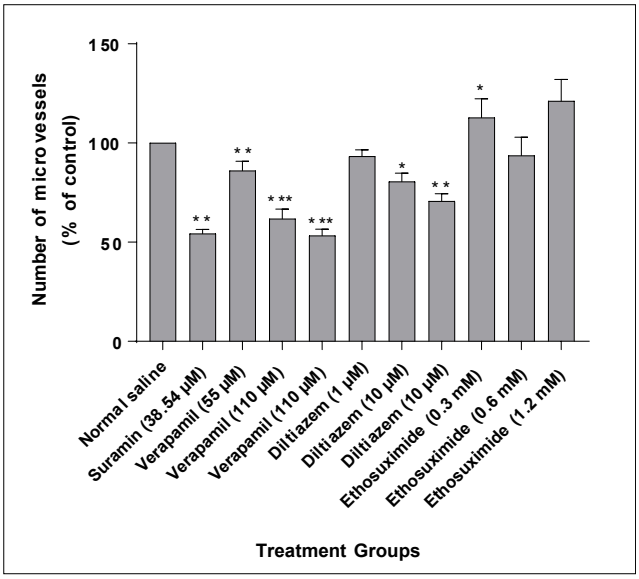


Figure 3. Comparative effect of L-type and T-type calcium channel blockers on the area of sprouts formed in aortic ring assay of angiogenesis.

4. Discussion

Angiogenesis is one of the critical steps in tumor progression and metastasis[22,23]. Pro- and anti-angiogenic factors regulate the formation of new blood vessels[22,24]. Excessive angiogenic happens when the effects of angiogenic factors become preponderant on the anti-angiogenic factors leads to formation of new blood vessels supplying tumor tissue[24].

Most mammalian cells represent numerous types of calcium channels involved in the regulation of the membrane potential the transport of osmolytes, and cell-volume regulation but the exact role of these channels in cell angiogenesis is not clearly defined. Earlier, voltage-dependent calcium channels blockers were used and have shown potential antiproliferative effects in several tissues but none of these drugs are clearly specific to one type of channel but they all highlight the role of calcium channels in the control of cell proliferation but not defined their exact role in angiogenesis[25]. Based upon these considerations in our study we have screened and compared the anti-angiogenic effect of voltage-dependent L-type (verapamil and diltiazem) and T-type (Ethosuximide) against CAM assay and aortic ring assay of angiogenesis. We report here that verapamil has shown the potent anti-angiogenic activity in both the models of angiogenesis in the dose-dependent passion. Diltiazem has also shown the activity but not as good as verapamil while ethosuximide has not shown any effect on the micro vessels formation. These results suggest that L-type calcium channels may play a role in the angiogenic cascade.

The CAM assay in the chick embryo is the most widely used vessel development assay in vivo. Initially, it was developed as a qualitative assay; ways were later reported for reliably quantifying the effects of promoters and inhibitors of angiogenesis on capillary development[26-27]. It is particularly suitable for the primary screening of potential inhibitors of angiogenesis in a living organism because it is simple and quick and vessels grow spontaneously with no need for the addition of external growth factors.

In the chick embryo, CAM shows the densest vascular networks so it can be considered that pro-angiogenic and angiogenic factors are present in the vascular growth.

The results our study represents that verapamil had shown significant ($p<0.001$) decrease in the number of capillaries formation, branching points and significant ($p<0.001$) increase in angiogenic score in all three doses in the dose-dependent manner (Figure 1, Figure 2 and Figure 4) compared to the control group. Moreover, the tested drugs with has shown anti-angiogenic activity the actions are mediated through the receptors because only drug-treated groups have shown the decline in blood vessel growth when compared to normal control group. Hypoxia and hence the free radical formation is one of the provoking factors for angiogenesis. In response to hypoxia, there is release of HIF (Hypoxia Inducible Factors) i.e. angiogenic and Vascular Endothelial Growth Factors (VEGF), which lead to the formation and growth of new blood vessels are considered as the basic and major inducers of angiogenesis. Based on these findings, we assumed two possible mechanisms.

In the blood capillaries, the formation of superoxide anions (O^{2-}) and free radicals considered as a vital factor responsible for endothelial dysfunction[29]. Verapamil

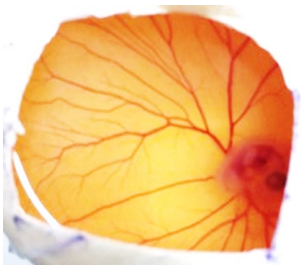
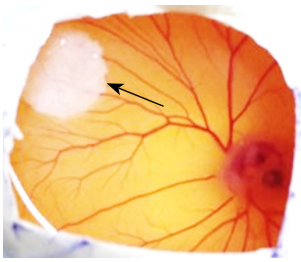
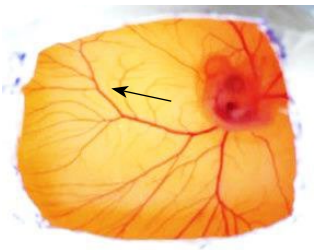
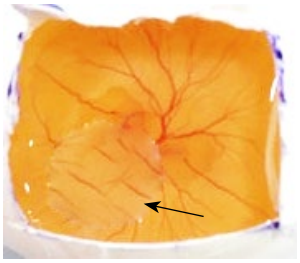
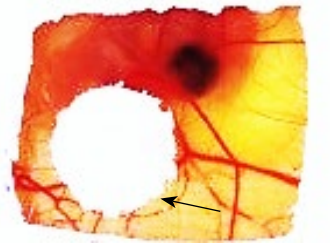
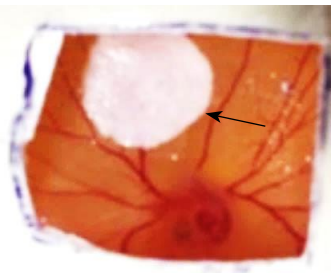
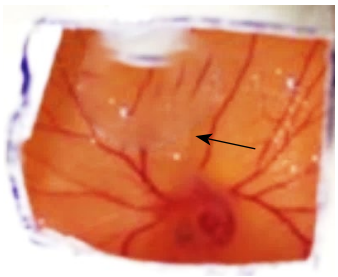
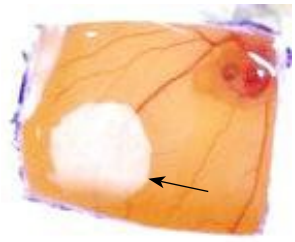
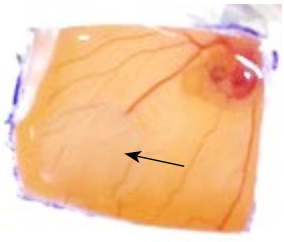
 Normal Control group	 Filter disk loaded with Distilled water	 Effect on the blood capillaries
 Before Suramin (38.54 µM) Treatment	 Filter disk loaded with Suramin (38.54 µM)	 Effect on the capillaries after Suramin (38.54 µM) Treatment
 Before Verapamil (55µM) Treatment	 Filter disk loaded with Verapamil (55µM) Treatment	 Effect on the capillaries after Verapamil (55µM) Treatment
 Before Verapamil (110 µM) Treatment	 Filter disk loaded with Verapamil (110 µM)	 Effect on the capillaries after Verapamil (110µM) Treatment
 Before Verapamil (220 µM) Treatment	 Filter disk loaded with Verapamil (220 µM)	 Effect on the capillaries after Verapamil (220µM) Treatment

Figure 4. Effect of L-type and T-type Calcium Channel blockers on neovascularization by using CAM assay of angiogenesis.

possesses antioxidant and free radical scavenging effects this actions of verapamil might have protected the endothelium from oxidative damage[28].

Second, as mentioned earlier in response to hypoxia there is a release of VEGF which induces angiogenesis via Receptor Tyrosine Kinases (RTK). Earlier literature shows that the verapamil reduces the VEGF formation and hence angiogenesis. The potency of antioxidant activity and VEGF inhibition activity is verapamil > diltiazem, this could be the possible reason why verapamil has shown better anti-angiogenic effect then diltiazem[28–30]. Ethosuximide has not shown any anti-angiogenic effect in both the models of angiogenesis. The possible explanation is that T-type calcium channels are predominantly present in the neuronal cells and those might be absent on the extra-neuronal tissue. Moreover, ethosuximide does not possess any effect on VEGF and free radicals, unlike verapamil and diltiazem.

Rat Aortic assay is another major model which was implemented in this research. In rat aortic ring assay, endothelial cells originate from the inner surface to from the capillary structure of rings of rat aorta which is immersed in the corneal matrigel. This assay also has many advantages like no need for repeated passages in culture to generate vascular out growths, (which resemble in-vivo angiogenesis), development of microvessels composed of endothelial cells and pericytes, self-controlling nature of the angiogenic response, external growth factor is not needed as they are naturally present in the developing embryo. The aortic ring assay provides a very good model which covers all major steps of angiogenesis like cell invasion, migration, proliferation, differentiation, and new vessel formation[31,32]. By the treatment with the verapamil at all three doses i.e., 55, 110 and 220 µM produced a drastic change in the area of sprouts formation in dose-dependent manner. (Figure 3 and Figure 5)

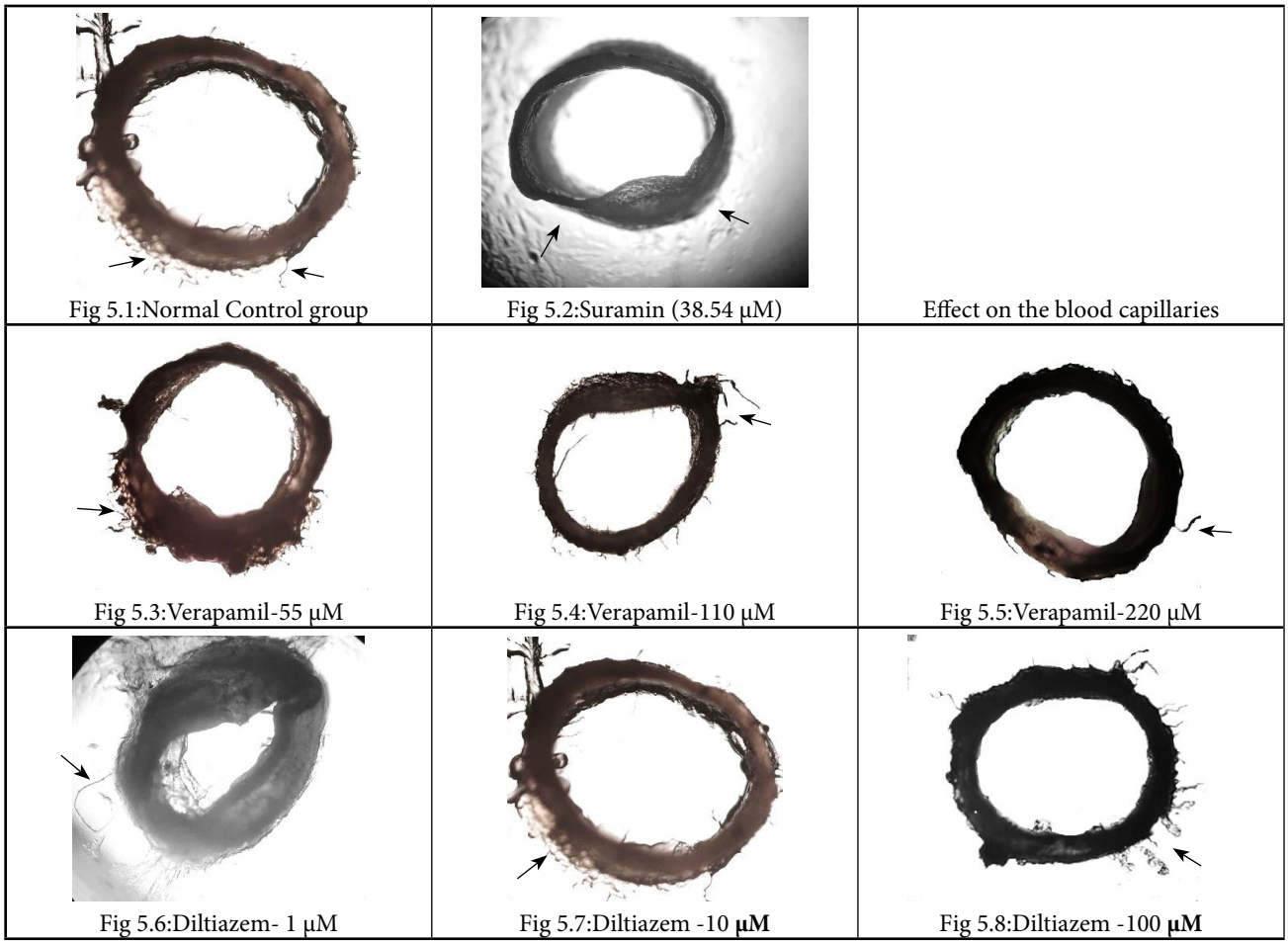


Figure 5. Effect of L-type and T-type calcium channel blockers on area of sprout formation by aortic ring assay method.

Ca^{2+} is one of the important signaling molecule involves in many physiological processes including muscle contraction, pacemaker activity, cell volume regulation, and is also an important component of regulation of cell cycle. So slight depletion or over loading then the normal limits of the intracellular calcium concentration may affect many physiological processes adversely. Therefore, overloading of calcium or disturbances in calcium signaling cause cell death[33–35].

Verapamil is an L-type calcium channel blocker. We found that verapamil in 55, 110 and 220 μM concentrations had a very good anti-angiogenic effect. However, diltiazem 1 and 10 μM concentration was a poor inhibitor of angiogenesis but at 100 μM shown good anti-angiogenic activity. The findings show that diltiazem has also an anti-angiogenic activity which is active and significant at high doses.

5. Conclusion

Based on our in-vitro studies we have shown that L-type calcium channel blockers are inhibitors of angiogenesis while T-type calcium channel blockers may not involve in cell proliferation and angiogenesis. Among the tested drugs verapamil has shown a very potent antiangiogenic activity as good as suramin. Our results reveal a probable physiological role for L-type calcium channel blockers in angiogenesis and suggest new insight to antiangiogenesis research by suggesting that L-calcium channel blockers play a key role in the angiogenic process and angiogenesis-dependent tumor growth. Verapamil and diltiazem are already in the clinical use for cardiovascular complications and considered as safe drugs. This additional new finding of these drugs will help prescriber to prescribe such drugs for the co-comorbid patients.

6. Acknowledgment

The author gratefully acknowledges the approval and the support of this research study by grant no. 5777-PHM-2016-1-6-F from the Deanship of Scientific Research at Northern Border University, Arar. K.S.A.

7. Conflict of Interest

Authors don't have any conflict of interest.

8. References

1. Folkman J, Klagsbrun M. Angiogenic factors. Science. 1987 Jan 23; 235(4787):442–7. crossref PMid:2432664
2. Folkman J. Seminars in medicine of the beth israel hospital, Boston. Clinical applications of research on angiogenesis. N Engl J Med. 1995 Dec 28; 333(26):1757–63. crossref PMid:7491141
3. Otrock ZK, Mahfouz RA, Makarem JA, Shamseddine AI. Understanding the biology of angiogenesis: Review of the most important molecular mechanisms. Blood Cells Mol Dis. 2007 Sep-Oct; 39(2):212–20. crossref PMid:17553709
4. Tahergorabi Z, Khazaei M. A review on angiogenesis and its assays. Iran J Basic Med Sci. 2012 Nov; 15(6):1110–26. PMid:23653839 PMCid:PMC3646220
5. Deryugina EI, Quigley JP. Tumor angiogenesis: MMP-mediated induction of intravasation- and metastasis-sustaining neovasculature. Matrix Biol. 2015 May-Jul; 44-46:94–112. crossref PMid:25912949 PMCid:PMC5079283
6. D'Amore PA, Thompson RW. Mechanisms of angiogenesis. Annu Rev Physiol. 1987; 49:453–64. crossref PMid:2436570
7. Augustin HG. Antiangiogenic tumour therapy: will it work? Trends Pharmacol Sci. 1998 Jun; 19(6):216–22. crossref
8. Balk SD. Calcium as a regulator of the proliferation of normal, but not of transformed, chicken fibroblasts in a plasma-containing medium. Proc Natl Acad Sci U S A. 1971 Feb; 68(2):271–5. crossref PMid:5277067 PMCid:PMC388915
9. Boynton AL, Whitfield JF, Isaacs RJ, Morton HJ. Control of 3T3 cell proliferation by calcium. In Vitro. 1974 Jul-Aug; 10:12–7. crossref PMid:4471173
10. Durham AC, Walton JM. Calcium ions and the control of proliferation in normal and cancer cells. Biosci Rep. 1982 Jan; 2(1):15–30. crossref PMid:7037065
11. Boynton AL, Whitfield JF. Different calcium requirements for proliferation of conditionally and unconditionally tumorigenic mouse cells. Proc Natl Acad Sci U S A. 1976 May; 73(5):1651–4. crossref PMid:1064038 PMCid:PMC430357
12. Boynton AL, Whitfield JF, Isaacs RJ, Tremblay RG. Different extracellular calcium requirements for proliferation of nonneoplastic, preneoplastic, and neoplastic mouse cells. Cancer Res. 1977 Aug; 37(8 Pt 1):2657–61. PMid:872093
13. Parsons PG. Selective proliferation of human tumour cells in calcium-depleted medium. Aust J ExpBiol Med Sci. 1978 Jun; 56(3):297–300. crossref PMid:101190
14. Paul D, Ristow HJ. Cell cycle control by Ca^{++}-ions in mouse 3T3 cells and in transformed 3T3 cells. J Cell Physiol. 1979 Jan; 98(1):31-9. crossref PMid:762200
15. Lokman NA, Elder AS, Ricciardelli C, Oehler MK. Chick Chorioallantoic Membrane (CAM) assay as an in

vivo model to study the effect of newly identified molecules on ovarian cancer invasion and metastasis. Int J Mol Sci. 2012; 13(8):9959–70. crossref PMid:22949841 PMCid:PMC3431839
16. Burgermeister J1, Paper DH, Vogl H, Linhardt RJ, Franz G. LaPSvS1, a (1-->3)-beta-galactan sulfate and its effect on angiogenesis in vivo and in vitro. Carbohydr Res. 2002 Sep 9; 337(16):1459–66. crossref
17. Krenn L, Paper DH. Inhibition of angiogenesis and inflammation by an extract of red clover (Trifoliumpratense L.). Phytomedicine. 2009 Dec; 16(12):1083–8. crossref PMid:19665361
18. AlMalki WH, Shahid I, Mehdi AY, Hafeez MH. Assessment methods for angiogenesis and current approaches for its quantification. Indian J Pharmacol. 2014 May-Jun; 46(3):251–6. crossref PMid:24987169 PMCid:PMC4071699
19. Fernandes G, Barone A, Dziak R. Effects of verapamil on bone cancer cells In Vitro. J Cell Biol Cell Metab. 2016; 3:013.
20. Caglar Y, Ali Cetin, Demirci F, Zubeyde AP, Tuba K, Ahmet A, Meral C, Ozlem K Y, Ismihan G. Anti-angiogenic effects of diltiazem, imatinib, and bevacizumab in the CAM assay. International Journal of Scientific and Research Publications. 2013; 3(8).
21. Gomora JC1, Daud AN, Weiergräber M, Perez-Reyes E. Block of cloned human T-type calcium channels by succinimide antiepileptic drugs. Mol Pharmacol. 2001 Nov; 60(5):1121–32. crossref PMid:11641441
22. Kerbel R, Folkman J. Clinical translation of angiogenesis inhibitors. Nat Rev Cancer. 2002 Oct; 2(10):727–39. crossref PMid:12360276
23. Fidler IJ. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat Rev Cancer. 2003 Jun; 3(6):453–8. crossref PMid:12778135
24. Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. Nat Rev Cancer. 2003 Jun; 3(6):401–10. crossref PMid:12778130
25. Capiod T. Cell proliferation, calcium influx and calcium channels. Biochimie. 2011 Dec; 93(12):2075–9. crossref PMid:21802482
26. Harris-Hooker SA, Gajdusek CM, Wight TN, Schwartz SM. Neovascular responses induced by cultured aortic endothelial cells. J Cell Physiol. 1983 Mar; 114(3):302–10. crossref PMid:6187756
27. Nguyen M, Shing Y, Folkman J. Quantitation of angiogenesis and antiangiogenesis in the chick embryo chorioallantoic membrane. Microvasc Res. 1994 Jan; 47(1):31–40. crossref PMid:7517489
28. Lam CF, Liu YC, Tseng FL, Sung YH, Huang CC, Jiang MJ, Tsai YC. High-dose morphine impairs vascular endothelial function by increased production of superoxide anions. Anesthesiology. 2007 Mar; 106(3):532–7. crossref PMid:17325512
29. Mason RP1, Mak IT, MW Trumbore, Mason PE. Antioxidant properties of calcium antagonists related to membrane biophysical interactions. Am J Cardiol. 1999 Aug 19; 84(4A):16L–22L. PMid:10480441
30. Hayman SR, Leung N, Grande JP, Garovic VD. VEGF inhibition, hypertension, and renal toxicity. Curr Oncol Rep. 2012 Aug; 14(4):285–94. crossref PMid:22544560 PMCid:PMC3746763
31. Nicosia RF, Ottinetti A. Growth of microvessels in serum-free matrix culture of rat aorta. A quantitative assay of angiogenesis in vitro. Lab Invest. 1990 Jul; 63(1):115–22. PMid:1695694
32. Nicosia RF, Villaschi S. Autoregulation of angiogenesis by cells of the vessel wall. Int Rev Cytol. 1999; 185:1–43. crossref
33. Mariot P, Vanoverberghe K, Lalevee N, Rossier MF, Prevarskaya N. Overexpression of an alpha 1H (Cav3.2) T-type calcium channel during neuroendocrine differentiation of human prostate cancer cells. J Biol Chem. 2002 Mar 29; 277(13):10824-33. Epub 2002 Jan 17. crossref PMid:11799114
34. Ciapa B, Pesando D, Wilding M, Whitaker M. Cell-cycle calcium transients driven by cyclic changes in inositol trisphosphate levels. Nature. 1994 Apr 28; 368(6474):875–8. crossref PMid:8159248
35. Choi DW. Ionic dependence of glutamate neurotoxicity. J Neurosci. 1987 Feb; 7(2):369–79. PMid:2880938